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(54) **Ensilaging vegetable matter**

(57) A process for the preservation of vegetable material by lactic acidification which comprises admixing the vegetable material prior to ensilaging the same, with bacteria capable of initiating lactic fermentation, and enzymes and/or bacteria adapted to degrade higher glucides into fermentable sugars used by the bacteria initiating the lactic fermentation. The enzymes comprise a defined cellulolytic complex of fungal origin. The bacteria comprise Gram −ve bacteria belonging to the family of Enterobacteriaceae type Erwinia or Pectobacterium; group Herbicola, of the new species *Enterobacter agglomerons*, which breaks down starch, but not maltose. This composition with an enzyme and/or bacteria base, is incorporated on a cereal support.

GB 2 046 567 A

SPECIFICATION

Process and product for preserving fresh vegetables and moist byproducts of th Agro-alimentary industry try

The present invention is related to an improved process for preserving and valorizing fresh vegetables so as to allow the same to be consumed in an appetizing form in successive amounts, as the need arises, after harvesting said vegetables. The invention also relates to a composition or product for carrying out said process

On account of the importance of the problems raised by the preservation of fresh vegetables, considerable research work has been conducted with a view to resolving the same by devising an appropriate technique; this research work has encountered a certain number of obstacles difficult to overcome.

The technique of ensilaging is based on the principle of preserving fresh vegetables during a more or less extended period of time within a closed, tight enclosure in an acid environment or medium with a view to preventing the proliferation of putrifying alkalizing and gas-producing germs; these germs will not develop in an acid medium. A satisfactory ensilaging method is difficult to devise, since a great number of obstacles are encountered when endeavouring to put such method to practical use, the most difficult problems to be overcome being the formation of fermentation products which are dangerous to the health of animals or the health of man when the meat and dairy products are consumed by the latter.

The mechanism to be devised is as follows: allowing the lactic bacteria contained in the medium to produce lactic acid from available soluble sugars, in such a manner that the pH of the medium may be thus lowered to a value of about 4. However in many cases the vegetables to be ensilaged do not contain a sufficient amount of fermentable sugars, and the proliferation only of the lactic bacteria, which are contained in the medium is insufficient, the pH thus not being lowered rapidly enough to the required value 4, while butyric and anaerobic bacteria develop and convert the residual sugars into butyric and acetic acid, carbon dioxide and hydrogen, the proteins being degraded into ammoniacal and other metabolic phases.

Various approaches have been devised with a view to overcoming these drawbacks. More particularly, according to one known process, a chemical acidification is effected by adding various acids. However this known method may be dangerous under certain conditions. According to another known method, a biological acidification is effected by adding highly glucidolytic bacteria. However this biological acidification process has been found to be difficult to carry out in practice.

According to other known methods, a raw material having a high content of glucide, such as molasses, is added to the ensilaged material. The results obtained when carrying out such methods in practice are not consistent, and the process involves considerable expense.

French Patent specification No. 2 361 828 discloses a process of preserving and valorizing fresh vegetables by lactic acidification, which comprises adding to the vegetables, prior to ensilaging the same, bacteria capable of initiating lactic fermentation, and adding a degradation agent for degrading higher glucides into fermentable sugars which are adapted to be used by the bacteria that initiate the lactic fermentation. This degradation agent is constituted by gram+ bacteria which initiate the fermentation of starch, glucose, mannitol (mannite), rahmnose, saccharose, amygdaline, arabinose, while not initiating the fermentation of maltose, inositol and sorbitol; the bacteria are VP+, oxydase+, catalase∓, nitrates∓, urea−, indol− and H_2S− bacteria. Said degradation agent may also comprise one or more enzymes capable of splitting polysaccharides, especially starches, pentosames and other complex polysaccharides, so as to produce fermentable sugars. More particularly, according to the abovementioned patent specification, a hemicellulolytic complex of fungic origin the main activity of which is a galactomannanasic activity and which has secondary xylanase, amylase and pectylase activities, as well as an amylase may be added to the fodder with a view to obtaining the formation of the maltose necessary for the production of lactic acid by the bacteria.

French Patent specification No. 2 390 908 discloses an improvement to the process according to French specification No 2 361 828. In the latter specification the enzyme is exoamylase of fungic origin which does not completely degrade starch, whereas the amylase used in accordance with French Specification No. 2,390,908 is an amylase of bacterial origin.

This enzyme (endoamyalase) acts on the liquefied starches and produces mainly alpha-D maltose and a small amount of alpha-D glucose said amylase being active in the pH range comprised between 5 and 8. French specification No 2 390 908 also discloses the use of amyloglucosidase which is substantially more active with respect to the amylose and amylopectin chains for producing alpha-D glucose. The enzymatic composition according to said specification also comprises a hemicellulolytic complex of fungic origin which acts on the polysaccaric constituents of the cellular membranes and the dextrins in the pH range comprised between 5.5 and 8.

Patent Application No: 34183/77 (Serial No) the contents of which are incorporated herein by reference, claims priority from the French Applications from which the aforementioned French Patent Specifications derive and describes and claims processes and compositions as hereinbefore discussed.

The present invention is aimed at providing an improved process and an improved product for ensilaging vegetables, the improvement residing inter alia in the provision of a novel enzymatic composition and in the provision of a novel bacterial composition. The enzymatic composition contains in addition to the enzymes indicated hereinabove a cellulolytic or cellulase complex capable of splitting the

beta links (1--3) and (1--4) of the polysaccharides which are not attacked by the prior enzymatic composition.

The above-mentioned complex is a cellulolytic complex of fungic origin which degrades the natural cellulose and the other polysaccharides contained in the cellular membranes.

This enzyme is active in the pH range comprised between 2 and 5. A synergy phenomenon exists between the cellulolytic complex and the hemicellulolytic complex, as regards the degradation of the vegetable structures.

On account of the environment of the cellulose in the vegetable its hydrolysis requires a plurality of enzymes, or one single enzyme having a plurality of functions, to wit:

- hydrolysis of the amorphous zones,
- swelling of the crystalline regions and disrupture of the hydrogen links so as to produce linear molecules,
- hydrolysis of cellobiose to produce glucose,
- transglucosylation of disaccharides and oligosaccharides, as well as disrupture of the irregular links of cellulose and its primary hydrolysis products.

The cellulolytic complex has been chosen on the basis of the above criteria after a great number of tests had been performed on various vegetables.

A cellulase is defined by its activity as expressed in International Units per gramme of product, on International Unit (I.U.) corresponding to the amount of enzyme which sets free, in the form of reducing sugar, the equivalent of one micromole of glucose per minute on a given cellulosic substrate.

The activity varies depending on the nature of the substrate used:

- when the activity is tested on "Whatman" (Registered Trade Mark) paper powder CC31, the result is C_1 negative,
- when the activity is tested on carboxymethylcellulose, the result is C_x activity.

The complex used in accordance with the present invention has a very high cellulolytic activity, as well as secondary activities of the hemicellulolytic type.

The high cellulolytic power is indicated by extremely high C_t and C_x activity values, and by cellobiase (beta-glucosidase) activity.

The activity of the C_1 enzymatic complex, defined in accordance with current hypotheses as being a compound having C_x activity to which an unknown factor is added, expresses in particular a power of solubilisation of cellulose.

C_1 is capable of degrading the native cellulose (crystalline cellulose having a polymerization rate (DP) higher than 3,500 glucose units) into amorphous cellulose.

The activity of the C_x enzymatic complex expresses in particular the reducing of power endoglucanase and exoglucanase. These enzymes mainly act on the cellulose fibres in the zones of reduced crystallinity and split the long glucose chains into smaller units.

The activity of beta-glucodiase expresses an action on the units obtained as a result of the action of C_x.

These different activities cannot be considered separately. There exists a synergistic interaction between C_1 and C_x as far as the degradation of these homopolymers is concerned. The enzymatic mechanisms C_1 and C_x lead to the production of cellobiase; the latter is then submitted to hydrolysis by a beta-glucosidase, thus producing two glucose units.

Apparently the hydrolysis reactions of cellulose comprise the stages schematically indicated herein-below:

Native cellulose	C_1	reactive cellulose
Reactive cellulose	hydrolytic C_x	cellobiose
Cellobiose	Hydrolytic beta-glucosidase	2 molecules of glucose

The C_1 comples solubilizes the crystalline cellulose (polymerization degree DP higher than 3,500 glucose monomers) and produces reactive cellulose (amorphous cellulose).

Under the action of the enzymatic C_x complex (endoglucanase and exoglucanase) the chains of reactive cellulose are split either inside the chain and randomly, in the case of endoglucanase, or starting from the non-reducing chain ends, in the case of exoglucanase.

These reactions, taken as a whole, produce cellobiose units.

These cellobiose units are hydrolysed by beta-glucosidase to produce two glucose molecules.

In the case of fungic cellulases according to the invention the enzymatic C_1 and C_x complex is capable of hydrolyzing the insoluble native cellulose, so as to produce glucose.

The cellulases according to the invention are chosen not only on the basis of the above-indicated general criteria, but also on the basis of considerations regarding the particular ensilaging medium envisaged, taking into account the polysaccharolytic behaviour of these cellulases.

This polysaccharolytic behaviour has been determined on two types of substrates:

a) – pure substrates;

b) – dehydrated substrates.

CASE OF THE PURE SUBSTRATES:

The activity of cellulases A, B, C, D, E, and F has been determined on various substrates at a given concentration in a 10^{-1}M acetate buffer medium.

The activity has been measured in accordance with the conventional standards, to wit, at pH 4.8 and 50°C, after hydrolysis during 20 minutes. The reducing power has been determined by the method using hexaferrocyanide.

TABLE I

SUBSTRATE (concentration) / ENZYMES (activity)	"Whatman"® paper	C.M.C.	Pectin	Xylane	Arabino galactane	Starch	Locus bean
	1%	1%	0.25%	0.25%	0.10%	0.25%	0.25%
	UI/g (C_1)	UI/g (C_x)	UI/g	UI/g	UI/g	UI/g	UI/g
A	400	6444	356	511	22	394	544
B	211	5660	322	611	33	100	300
C	266	1999	411	311	0	244	389
D	52	6440	255	511	0	120	132
E	372	8500	305	605	0	0	0
F	960	7666	1030	1200	0	0	140

CASE OF THE DEHYDRATED SUBSTRATES:

The activity of enzymes A, B, C, D, E and F, has also been determined on various dehydrated vegetable substrates.

The activity has been tested at pH values of 3.8, 4.8 and 5.8 et 30°C after hydrolysis during 24 hours. The reducing power has been determined by means of the method using dinitrosalicyclic acid.

TABLE II

Tested cellulases / Tested substrates	pH	Lucerne	Ray-grass (Rye-grass)	Maize	Beetroot	Straw
		ACTIVITY (UI/g)				
A	3,8	2810	2280	1560	1810	1560
	4,8	2440	2560	2080	1530	1690
	5,8	2560	2220	1690	1500	1530
B	3,8	1560	1670	1530	1220	1390
	4,8	1280	2060	1670	1220	1500
	5,8	890	1680	1940	890	1380
C	3,8	1280	1530	1390	1110	1110
	4,8	1280	1750	1530	1110	1290
	5,8	940	1560	1170	720	1110
D	3,8	1390	1440	1470	1278	1167
	4,8	1190	2060	1390	1111	1306
	5,8	890	1720	1530	972	1222
E	3,8	611	1190	1111	556	694
	4,8	972	1528	1250	0	694
	5,8	0	1167	1278	0	694
F	3,8	1306	500	694	556	
	4,8	889	972	1222	610	444
	5,8	750	1000	560	550	333

The analysis of these various behaviours allowed the of the c llulolytic complex to be used in accordance with the invention to be used for ensilaging vegetables:

It was thus found that the cellulase or the cellulolytic complex to be added to the fresh vegetables when carrying out the process according to the invention must correspond to a C_1 activity of 50 to 0.005 I.U. and a C_x activity of 500 to 0.05 I.U. per 100 grammes vegetable, these activities being determined at pH 4.8 at a temperature of 50°C after hydrolysis for 20 minutes.

Furthermore, the selected cellulase must retain a considerable activity even after a long dwelling time in the silo.

It has been shown experimentally that under conditions close to those prevailing when vegetables are ensilaged the cellulolytic complex must retain at least 30% of its initial activity after 10 days.

TABLE III

Preservation	0	5	10	15	20
time (days)					
pH 4,8	100%	97%	61%	26%	3%
pH 5,8	100%	85%	58%	27%	0%

The velocity, or rate, of enzymatic degradation of the vegetable structure is a function of glucose elimination; this enzymatic unbalance is obtained by using bacteria having a high fermentation power; the acidification of the medium occurs very rapidly. When the vegetable mass has been stabilized the bacterial proliferation and the formation of lactic acid are discontinued, contrary to the cellulolytic activity of cellulase, which continues for more than 10 days.

This activity of producing and accumulating the glucose formed within the ensilaged material increases the nutritive value of the ensilaged vegetables.

The enzymatic composition used in accordance with the present invention thus comprises a cellulase or cellulolytic complex having the above-indicated characteristics, in association with the enzymes described in French Patent specification No 2 390 908, to wit, a fungic amylase, a bacterial amylase, an amyloglucosidase and a hemicellulolytic complex.

These enzymes present in the composition thus exhibit a complementary activity on account of their effect on the glucides – which may be extremely simple glucides or extremely complex ones – in pH ranges comprised between 7 and 2. The respective maximum activities of the various enzymes occur in relays as the pH decreases (which latter does not reach values lower than 3.5 in the silo), and within temperature intervals varying from 10 to 30°C which are compatible with the conditions prevailing in the silo.

The bacterial composition used in accordance with the present invention is characterized by the utilisation of novel bacterial strains adapted to be used when ensilaging vegetables, said strains being adapted to be used separately or in admixture with the bacterial strains and/or the enzymes disclosed in the prior art and in the present description. This novel composition leads to considerably improved ensilaging results. The novel bacterial strains according to the invention are gram – bacteria; they belong to the Enterobacteriaceae family, the Erwinia or Pectobacterium type and the Herbiola group; they are called Enterobacter agglomerans (classification according to Bergey's Manual of Bacteriology, eighth edition).

The properties of such bacteria are listed in the appended TABLE IV.

TABLE IV

Enterobacter Agglomerans		Adonitol	–
Gram	–	Inositol	–
Utilisation malonate	+	Sorbitol	–
Glucose	+	Arabinose	+
Phenylalamine desaminase	–	Maltose	–
ONPG	+	Trehalose	+
Indole	–	Xylose	–
H_2S	–	Starch	+
LDC	–	Oxydase	–
ODC	–	Gelatine	+
Urease	–	Amygdaline	+
Saccharose	+	Melibiose	–
Arginine dihydrolase	–	V.P.	+
Salicine	–	Rhamnose	–

The utilisation of these novel bacterial strains, separately or in combination with other bacterial strains and/or enzymes, for ensilaging vegetables is effected in accordance with the operating conditions described in the prior publications. The bacteria may be grown on a nutritive support comprising cereal wheats. The composition to be introduced into the silo comprises a mixture of the required bacteria deposited on a soluble or insoluble support, with the enzymes defined in the present disclosure.

In a preferred embodiment of the present invention, the enzymes and the bacteria are placed on a cereal support (corn, barley, germinated barley), preferably in a finely ground condition.

In another preferred embodiment of the invention the bacteria and the enzymes are placed onto a support of a type constituted by pre-gelated starch, maltohexose, etc..., which support is highly soluble in cold water.

The starch and other polysaccharides contained in the supports combine with the glucides of the vegetables and thus increase the nutritive value of the ensilaged matter. The composition comprising the mixture of bacteria on their support with the enzymatic complex, deposited or not on a cereal support, may include various proportions of each of one of these constituents. The dry mixture comprises an amount of about 100,000 to 1 million cocci and 100,000 to 1 million bacilli per gramme.

The amounts of cellulase, hemicellulolytic complex, amylase and amyloglucosidase may vary; they are calculated according to the nature of the fodder to be treated.

The amount of cellulase to be added to the ensilaged fresh vegetables must correspond to a C_1 activity of 50 to 0.005 I.U. per 100 g. vegetable; it must thus be equal to 2 to 2.10^{-4} ‰by weight of fresh vegetables when a cellulase having a 250 I.U./gramme titre is used. The proportions of the other enzymes are comprised between 0.05 and 0.20‰ bacterial amylases having a 250 units/gramme titre (by weight of vegetables), 0.10 to 0.20‰ fungic amylases having a 50,000 units AG/gramme titre (by weight of vegetables), 0.10 to 0.70‰ amylglucosidase having a 200 units AG/gramme titre (by weight of vegetables), 0.05 to 0.20‰ hemicellulolytic complex having a 35,000 I.U./gramme titre (by weight of vegetables).

When the enzymes are deposited on a cereal support in accordance with a preferred embodiment of the present invention, the product to be incorporated in the ensilaged matter may contain about 1 kg enzymes per 9 kg support. When a soluble support is used, the ratios of enzyme concentration to support vary and may be comprised between 1/1.5 and 1/4.

Due to this incorporation of cellulase in the support, the amount of available fermentable sugars is increased, whereby a larger and more rapid production of lactic acid is obtained. The vegetable protein wil thus be more efficiently preserved. Furthermore, the nutritive value of the medium will be increased.

With a view to showing the advantages brought about by using a cellulolytic complex and bacteria of the Enterobacteriaceae family, tests were performed in micro-silos and mini-silos. The various preservation parameters as well as the vegetable structure parameters were studied.

1. *TESTS PERFORMED IN MICRO-SILOS*

A first laboratory test series was performed in micro-silos. These tests were carried out in May on lucerne (alfafa). The micro-silos had a capacity of 1 kg. The lucerne was finely chopped and then ensilaged in accordance with the following operating mode:

A – One series of micro-silos were treated by means of a preserving additive having the following general formula:

cereal support	90%
pre-mixture	10%,

the pre-mixture being constituted by:

- a bacterial complex (5 lactic bacteria on lactose support):
- an enzymatic complex comprising:
  - . an amylase of fungic origin;
  - . an amylase of bacterial origin;
  - . an amyloglucosidase;
  - . a hemicellulolytic complex of fungic origin, the main activity of which was a galactomannasic activity;
- a high-energy support : finely ground barley + lactoserum, the bacterial and enzymatic complexes representing each 1% of the pre-mixture composition.

The preservation additive was admixed with the lucerne in a proportion of 2% (the rate of incorporated pre-mixture thus being 0.2%), and 1% barley was added.

B – A series of micro-silos were treated with the same preservation additive as that used according to A, however, a cellulase of fungic origin (stemming from Trichoderma viride) was added, said cellulase presenting a C_1 activity of 250 units/gramme and a C_x activity of 2,500 units/gramme, as determined at pH. 4.8 at a temperature of 50°C, for a reaction time of 20 minutes, the proportion of incorporation of this cellulase being 2 g/T (i.e. 2.10^{-4}% by weight) as referred to the fresh vegetables (Test B) and 200 g/T (i.e. 0.02%) by weight as referred to the fresh vegetables (Test B').

C – A series of micro-silo reference tests:

- a series of micro-silo tests with lucerne containing no preservation product : T = O.

After 100 days duration of ensilaging the micro-silos were opened, the following parameters were recorded : pH, lactic acid, NH_3/N (total). The results are listed in TABLE V overleaf.

It can be seen that the admixing of a cellulase to a bacteria + enzyme complex according to patent specification no 2 390 908 leads to an improvement of the performances of the preservation additive. A more satisfactory preservation of the vegetables is obtained, as illustrated by the comparison of A and B', as follows:

- a decrease of the pH value from 4.13 to 3.73 (decrease of 10%);

TABLE V

	pH	Lactic acid (g/kg MS)	Total NH_3/N
Référence: T = 0	5.66	26g	25.15
. Premixed lucerne + 0,2%(A).	4.13	143.68	16.33
. Premixed lucerne + 0,2% + cellulase of fungic origin 2 g/T (B).	4.32	144.75	16.20
. Premixed lucerne + 0,2% + cellulase of fungic origin 200 g/T (B')	3.73	169.47	15.02

- a 15% decrease of the lactic acid production;
- a 8% decrease of the total NH_3/N ratio; this shows an improvement of the preservation of the vegetable protein which results from the rapid acidification of the medium.

2. *TESTS PERFORMED IN MINI-SILOS*

These tests were carried out in June with lucerne in mini-silos having a capacity of 150 kg.

The lucerne was finely chopped, then ensilaged in accordance with the operation conditions indicated herein-above.

A. – A series of mini-silos was treated by means of the same preservation additive as that used in the micro-silo tests described under A, the proportion of incorporation being 0.2% pre-mixture.

B. – A series of mini-silos was treated in accordance with the formula and the dose indicated under A, to which the same cellulase of fungic origin was added in a proportion of 2 grammes/T.

After 100 days ensilaging the mini-silos were opened, the analysis of the ensilaged materials gave the results shown in TABLE VI overleaf.

The results listed listed herein-above clearly show the advantages of adding a cellulase to the bacterial + enzyme complex described in Patent specification no 2 390 908.

This addition results in:

- a decreased pH : 3.94 as compared to 4.20 (less 6%);

TABLE VI

	A silos 0.2% premixture	B silos 0.2% premixture + cellulase (2 g/T)
. Dry matter (MS) %	23.67	27.27
. E.N.A. (g/kg MS)	51.25	48.97
. pH	4.20	3.94
. Lactic acid (g/kg MS)	80.62	114.40
. Acetic acid (g/kg MS)	17.42	20.04
. Total NH_3/N	18.40	13.31
. Total N (g/kg MS)	21.70	23.12
. Ammoniacal N(g/kg MS)	3.87	3.09

- an increased proportion of lactic acid (plus 29.53%);
- a more satisfactory preservation of the proteinic part of the ensilaged matter, when a cellulase is added; this is confirmed by analysis.

The following results are obtained in this case:

- a decreased total NH/N ratio (less 27.6%);
- an increased proteic rate (plus 6%);
- a decreased ammoniacal nitrogen rate (less 20.1%).

This increased acidification velocity may be explained by the fact that the bacteria are provided more rapidly with an important amount of fermentable sugars which are easily converted into lactic acid. The effect of the cellulase introduced into the medium can be seen here again.

In the examples given herein-below, the mixture applied on the support in accordance with the invention was constituted by the complex described in French Patent Specification no 2 361 828 and/or in French Patent Specification no 2 390 908, the enzymatic complex disclosed in the present description, to which gram — bacilli of the Enterobact riaceae family, of the Erwinia or Pectobacterium type, Herbicola group, were added, the proportion of incorporation of these bacilli in the mixture corresponding to 10^5 to 10^6 bacilli per gramme

EXAMPLES

Tests were carried out in silos having a capacity of 4 m^3 with Dactile Lucifler harvested in June, at the starting stage of heading. The finely chopped fodder was stocked under four different conditions:

A. ensilaging without treatment;
B. ensilaging with 4 litres formic acid/ton in accordance with known methods;
C. ensilaging with the pre-mixture, the composition of which is described in French Patent specifications no 2 361 828 and no 2 390 908: ensilaging the matter to be treated with an addition of 7 kg of the strain of 7 bacteria on support, the properties of which are indicated in French Patent Specification no 2 361 828 page 7, no 1 to 7 and enzymes; proportion of incorporation: 10 kg/ton;
D. ensilaging with the pre-mixture according to C, which bacilli according to the invention were added, to wit: gram – bacteria, bacteria of the Enterobacteriaceae family, Erwinia or Pectobacterium type, Herbicola group, named Enterobacter agglomerans. The proportion of the incorporation of the complex was 10 kg/T.

After 90 to 100 days the different silos were opened, and the vegetables were analysed. The results thus obtained were listed herein-after in TABLE VII.

The results show that the mixture according to the French Patent specifications no 2 361 828 and no 2 390 906 led already to a considerable improvement, as regards the preservation of the ensilaged matter, which is illustrated by:

TABLE VII

Parameters / Treatments	pH	$\frac{N\text{–}NH_3}{\text{Total N}}$ %	$\frac{\text{N soluble}}{\text{Total N}}$	Organic acids (g/kg M.S.)			
				lactic	propionic	acetic	butyric
A : not treated.	5.28	17.76	63.80	23	1.38	29.46	traces
B : treatment with formic acid 4%.	4.05	7.10	45.43	41.36	traces	14.95	0
C : treatment according French Patent 2.390.908.	4.02	14	48.10	90	0	14.20	0
D : novel treatment (C=bactericid straties according to the invention).	3.70	8	44	112	0	18.28	0

- a decrease of pH value, which was lowered from 5.28 to 4.02;
- an increased lactic acid content of 90 g/kg MS as compared to 23 g/kg MS;
- an improved proteinic protection which is illustrated by decrease of the $\frac{N\text{–}NH_3}{\text{total N}}$ ratio dropping from 17.76 to 14 and of the $\frac{\text{soluble N}}{\text{total N}}$ ratio dropping from 63.80 to 48.10.

This mixture had the advantage of leading to no formation of propionic and butyric acid.

The mixture according to the present invention resulted in a supplementary improvement which is illustrated by:

- a decrease of the pH value (dropping from 4.02 to 3.70); an increased lactic acid content (112 g/kg MS as compared to 90 g/kg MS);
- an improved proteic protection which is illustrated by a decrease of the $N-NH_3$/total N ratio (dropping from 14 to 8 and of the soluble N/total N ratio (dropping from 48.10 to 44).

One advantage of this mixture resides in the fact that it leads to no fermentation of propionic and butyric acid.

The digestibility and ingestibility of these ensilaged matters were measured on mutton; after the various treatments the following results were found:

B : treatment with formic acid : 0.64 UF/kg;

C : treatment according to French Patent specification no 2 361 828 and no 2 390 908 : 0.74 UF/kg;

D : treatment according to the present invention : 0.82 UF/kg.

The nitrogen balance on animals in the growth period produced results which showed the advantages brought about by the treatment with ensilaging products according to the present invention, as illustrated in TABLE VIII.

The nitrogen retention obtained when applying the method according to the present invention is more than twice as large as that obtained by treatment with formic acid.

The invention is not limited by the foregoing description and the various embodiments illustrated therein. Various modifications may be envisaged by those skilled in the art without departing from the spirit and the scope of the invention as defined by the appended claims.

The Enterobacter Agglomerans bacteria employed in embodiments of the present invention has been lodged at the C.N.C.M., Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris, Cedex 15, France on 11th February 1980, under accession number 1-114.

TABLE VIII

Parameters / Treatments	Ingested N g/j	Fecal N g/j	Fecal N Ingested % N	Urinary N g/j	Urinary N Ingested % N	Retained N g/j	Retained N Ingested % N
A : not treated.	21	7.90	3.76	13	61.9	0.10	0.50
B : treatment with formic acid 0.4%.	18.9	7.34	38.8	10.4	55	1.1	5.88
C : treatment according to main patent + 1st Addition.	17	7.07	41.6	8.12	47.7	1.8	10.50
D : treatment according to invention.	16.5	5.4	38.8	7.70	47	2.40	14.50

CLAIMS

1. A process of preserving and valorizing fresh vegetable matter by lactic acidification, comprising admixing to said vegetable matter prior to ensilaging the same, bacteria capable of initiating lactic fermentation, and adding degradation agent which is adapted to degrade higher glucides into fermentable glucides for use by the bacteria initiating said lactic fermentation, and which is constituted by enzymes and/or bacteria, wherein said enzymes if present, comprise in combination with a fungic amylase, an amyloglucosidase, a hemicellulolytic complex of fungic origin having primarily a galactomannanasic activity, a native glucose cellulose having a C_1 activity of 50 to 0.005 International Units and a C_x activity of 500 to 0.05 International Units per 100 grammes fresh vegetables and retaining under the prevailing ensilaging conditions at least 30% of said activity after 10 days, and said bacteria, if present, being gram-bacteria which initiate starch fermentation but do not initiate maltose fermentation, and belonging to the family of Enterobacteriaceae, type Erwinia or Pectobacterium, group Herbicola, named Enterobacter agglomerans.

2. A process according to Claim 1, wherein the bacteria belonging to the family of Enterobacteriaceae are used in association with other bacteria capable of degrading higher glucides into fermentable glucides.

3. A process according to claim 2, wherein said other bacteria are selected from the bacteria capable of degrading higher carbohydrates which are disclosed in Patent Application No: 34183/77 (Serial No. . . . .).

4. A process according to claim 1 or 2, wherein the bacteria belonging to the family of Enterobacteriaceae are used in association with gram + bacteria which ferment starch, glucose, mannitol, rahmnose, saccharose, amygdaline and arabinose and do not ferment maltose, inositol and sorbitol.

5. A process according to any preceding claim, wherein the enzymes are present as a mixture cons-

tituted by:

- 2.10^{-4} to 2‰ cellulose having a titre of 250 I.U. C_1/g,
- 0.05 to 0.20‰ bacterial amylase having a titre of 250 units/g,
- 0.10 to 0.20‰ fungic amylase having a titre of 5,000 units P.S. 50/g,
- 0.10 to 0.70‰ amyloglucosidase having a titre of 200 units AG/g,
- 0.05 to 0.20‰ hemicellulolytic complex having a titre of 35,000 I.U./g,

the percentage values being expressed by weight with reference to the ensilaged vegetables.

6. A process according to any one of the preceding claims, wherein the enzymes are present and are incorporated on a cereal support in a proportion of about 1 kg enzymes per 9 kg support.

7. A process according to any one of the preceding claims, wherein the bacteria are present and are cultivated on a support comprising cereal wheats.

8. A process according to any one of claims 1 to 5, wherein the bacteria and the enzymes are present and are incorporated on a support of the cereal starch type, pregelated starch type, malto-hexose type, which is soluble in cold water, in a proportion of 1 kg bacteria and enzymes per 1.5 to 4 kg support.

9. A process according to one of the preceding claims wherein the amount of enzymes and bacterial culture with their support, in the dry state, added per ton of vegetables to be ensilated is 10 to 15 kg, this additive containing 10^5 to 10^6 live germs per gramme.

10. A composition for preserving and valorizing fresh vegetables which comprises lactic acid-producing bacteria strains and a degradation agent capable of degrading higher glucides into fermentable glucides constituted by an enzymes complex and/or bacteria, wherein the enzymes if present comprise, possibly in association with fungic amylase, amyloglucosidase, a hemicellulolytic complex of fungic origin having primarily a galactomannanasic activity, a cellulase or cellulolytic complex of fungic origin, capable of degrading the native cellulose into glucose and having a C_1 activity of 50 to 0.005 International Units and a C_x activity of 500 to 0.05 International Units for 100 grammes of fresh vegetables and retained under the prevailing ensilaging conditions at least 30% of said activity after 10 days, said bacteria if present, being gram-bacteria which initiate starch fermentation but do not ferment maltose, belonging to the family of Enterobacteriaceae, type Erwinia or Pectobacterium, group Herbicola, named Enterobacter Agglomerans.

11. A composition according to claim 9, wherein the Enterobacteriaceae gram-bacteria are present and are associated with bacteria capable of degrading the higher glucides into fermentable sugars, and especially gram + bacteria which ferment the starch, glucose, mannitol, rahmnose saccharose, and amygdaline, arabinose, and do not ferment maltose, inositol and sorbitol.

12. A process of preserving and valorizing fresh vegetable matter by lactic acidification, as claimed in claim 1 and substantially as hereinbefore described, with reference to any one of runs B and B' of Test 1, run B of Test 2, and run D of the Examples.

13. A composition for preserving valorizing fresh vegetable matter, as claimed in claim 10 and substantially as hereinbefore described with reference to any one of runs B and B' of Test 1, run B of Test 2 and run D of the Examples.

14. Vegetable matter when treated by a process according to any of claims 1 to 9 and 12 or by means of a composition, according to any of claims 10, 11 and 13.

15. An animal fed upon vegetable matter as claimed in claim 14.

16. The features hereinbefore disclosed, or their equivalents, in any novel selection.

New claims or amendments to claims filed on 28 July 1980.
Superseded claims 1.

1. A process of preserving and valorizing fresh vegetable matter by lactic acidification, comprising admixing to said vegetable matter prior to ensilaging the same, bacteria capable of initiating lactic fermentation, and adding degradation agent which is adapted to degrade higher glucides into fermentable glucides for use by the bacteria initiating said lactic fermentation, and which is constituted by enzymes and/or bacteria, wherein said enzymes, if present, comprise, optionally in combination with a fungic amylase, with an amyloglucosidase, or with a hemicellulolytic complex of fungic origin having primarily a galactomannanasic activity, a cellulase or cellulolytic complex of fungic origin capable of degrading the native cellulose into glucose and having a C_1 activity of 50 to 0.005. International Units and a C_x activity of 500 to 0.05 International Units per 100 grammes fresh vegetables and retaining under the prevailing ensilaging conditions at least 30% of said activity after 10 days, and said bacteria, if present, being gram-bacteria which initiate starch fermentation but do not initiate maltose fermentation and belonging to the family of Enterobacteriaceae, type Erwinia or Pectobacterium, group Herbicola, named Enterobacter agglomerans.

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